Current Status of Therapy of Solid Tumors

Yuliya S. Jhanwar, MD and Chaitanya Divgi, MD

Department of Radiology, Memorial Sloan-Kettering Cancer Center, New York, New York

The recent approval of 2 radiolabeled antibodies against cluster designation 20–positive lymphoma has led to a resurgence of interest in radioimmunotherapy. As was the case with chemotherapy development, progress has been most marked in the hematologic neoplasms, both in myeloablative and in nonmyeloablative therapeutic strategies. Success in the radioimmunotherapy of solid tumors has lagged because of the immunogenicity of murine proteins and the relatively slow clearance of humanized intact immunoglobulins. Genetic engineering has enabled the development of a variety of antigen-binding constructs of various sizes and immunobiologic characteristics. Developments in radiochemistry as well as production of an increasing number of radionuclides with therapeutic potential or optimal imaging characteristics have spurred tailored therapeutic strategies that include dosimetry and considerations of tumor burden. Such progress has generated pivotal studies that will establish the radiobiologic paradigms for successful radioimmunotherapy of solid tumors. This review will describe seminal studies that have paved the way to an understanding of radioimmunotherapy in solid tumors. Finally, the authors' views of the future of this promising cancer therapy will be presented.

Key Words: radioimmunotherapy; solid tumors; antigen-binding construct; radionuclides

J Nucl Med 2005; 46:141S–150S

Although it has been only 2 years since the Food and Drug Administration (FDA) approved the use of the first radiolabeled antibody, radioimmunotherapy (RIT) has been the subject of investigations since the early 1970s. As one of many promising therapeutic strategies that are encompassed by the magic-bullet concept, RIT uses an antibody that recognizes tumor-associated antigens and carries cytotoxic radionuclides to a target, thereby destroying cancer cells. The approval of 2 radiolabeled antibodies in the treatment of non-Hodgkin's lymphoma has offered hope about the role of RIT in the management of cancer. Nevertheless, the development of effective RIT faces many challenges. The success of RIT thus far has been driven by the results seen in hematologic tumors, whereas solid tumors have been less responsive.

Currently, the only radiolabeled antibodies with FDA approval are for the treatment of B-cell non-Hodgkin's lymphoma. Several trials of various antibodies for the treatment of non-Hodgkin's lymphoma have demonstrated a satisfactory response in relapsed and refractory disease, as well as potential for use with certain chemotherapeutic agents and autologous stem cell transplantation (1–5). The results of RIT for solid tumors have been less encouraging. Response to RIT has been studied in various tumor types, including breast, ovary, colorectum, kidney, prostate, and brain, although no response has been sufficiently promising to warrant further development with nonmyeloablative RIT. The initial clinical trials on solid tumors have, however, shed considerable light on antigen-binding construct design, optimal radionuclides for tumors of various sizes, and treatment delivery systems that will result in novel approaches to targeted radiopharmaceutical therapy.

Designing optimal RIT for solid tumors depends on many variables. Choosing a construct for solid tumors, which are generally large and heterogeneous, is of particular concern. Factors to consider when designing therapy include immunogenicity, heterogeneity of antigen expression, uptake by tumor secondary to vascular and tumor permeability, deleterious effects on normal tissues, and residence time in the body (6,7). Recombinant techniques, as well as the development of small antigen-binding constructs, are just some of the methods being used to address the optimal targeting of a tumor.

The choice of the optimal construct and radionuclide is part of the challenge of designing therapy for solid tumors that is the most effective and that offers the fewest limitations. An understanding of RIT therefore requires an understanding of the potential and limitations of radionuclides and antigen-binding constructs. These will be outlined, followed by proposed strategies for rational treatment designs that address the biologic complexity of solid tumors.

ANTIBODY CONSTRUCTS

The move from polyclonal antibodies to murine monoclonal antibodies (mAbs) brought into focus the inherent immunogenicity of xenogeneic proteins. The production of human antimouse antibodies after murine mAb administration precludes effective multiple-dosing regimens. The use of chimeric antibodies, in which a gene for the human immunoglobulin constant region is fused with the murine variable (Fv) region, has significantly reduced immunoge-

TABLE 1
Therapeutic Radionuclides and Their Half-Lives, Energy, and Range in Tissue

Radionuclide	Half-life	Energy
^{131}I*†	8 d	β_{ave} 191 keV
^{90}Y†	2.67 d	β_{ave} 934 keV
^{177}Lu*	6.7 d	β_{ave} 150 keV
^{186}Re*	3.78 d	β_{ave} 362 keV
^{188}Re*	17 h	β_{ave} 795 keV
^{67}Cu*	61.8 h	β_{ave} 141 keV
^{211}At*	7 h	7.5 MeV (^{211}Po)
^{213}Bi*	46 min	8.4 MeV (^{213}Po)
^{225}Ac	10 d	8.4 MeV

*Photon.
†$\beta+$ isotope.
β_{ave} = average β-energy.
Data are from http://ie.lbl.gov/toi/nucSearch.asp.

nicity. Humanized antibodies are generated from the gene of the antigen-binding or complementarity-determining region on a human IgG background. These are also used as a less immunogenic alternative to murine antibodies. Development of these types of antibodies will be an essential part of treatment regimens that require multiple administrations for optimal response, although the development of human antihuman antibodies has been seen occasionally.

One method of improving tumor penetration and clearance from normal tissues is the use of small constructs. Fab′ and F(ab)′₂ are 2 such constructs that are being investigated in mouse models and humans with good results. Single-chain antigen binding proteins (sFv) are linear constructs of light and heavy Fv fragments that clear rapidly from the blood and may have lower renal retention than do Fab′ fragments. Other constructs with rapid clearance are minibodies, which consist of 2 sFv fragments linked by a component of the heavy-chain region (e.g., CH₃), and diabodies, which comprise 2 sFv fragments joined chemically by disulfide bonds or by genetic engineering of the sequences.

RADIONUCLIDES

A major consideration in the development of RIT is the choice of radionuclide. Characteristics such as physical and chemical properties, fate after antibody metabolism in vivo, and the nature of the radiation are determinants of the suitability of a radionuclide for therapy. Cytotoxic radionuclides may be divided into 3 groups of radiochemicals: halogens (iodine, ^{211}At), metals (^{90}Y, ^{67}Cu, ^{213}Bi, ^{212}Bi), and transitional elements (^{186}Re). Radionuclides can further be categorized into 4 types of cytotoxic agents: pure β-emitters (^{67}Cu, ^{90}Y); α-emitters (^{213}Bi, ^{211}At), β-emitters that emit γ-radiation (^{177}Lu, ^{186}Re, ^{131}I), and Auger emitters and radionuclides that decay by internal conversion, including ^{125}I and ^{67}Ga (Table 1).

^{131}I is the most widely understood radionuclide for the treatment of cancer. ^{131}I is a mainstay of the treatment of thyroid cancer, and thus its biodistribution has been well studied. ^{131}I offers the advantages of high-energy γ-emissions for imaging, long half-life, and a simple protein labeling process. Although the high-energy γ-emissions have created potential concerns about the safety of patients and their contacts, recent guidelines will permit many patients receiving RIT with ^{131}I to be treated as outpatients. Hospitals in the United States may release patients only if the expected exposure to an individual due to proximity to the patient is no greater than 5 mSv (500 mrem) (8).

^{131}I is not the optimal nuclide when conjugated with antibodies that are internalized via the lysosomal pathway after the antibody–antigen interaction. This process leads to dehalogenation of the complex and release of the radionuclide, thus limiting tumor dose. Because of these limitations, other radionuclides have been studied. ^{90}Y, a pure β-emitting metal, has been widely studied as a high-energy alternative to ^{131}I. ^{90}Y is an attractive choice for RIT involving internalized antibody–antigen complexes, as radiometal antibodies are infrequently degraded internally. The success of ^{90}Y for use in RIT is evident, as the first RIT to be approved by the FDA was an anti–cluster designation 20 antibody conjugated to ^{90}Y (Zevalin; Biogen Idec Pharmaceuticals). One limitation of yttrium, however, is its affinity for bone. In a situation in which ^{90}Y is detached from the chelating agent, bone marrow could thus receive unacceptably high levels of radioactivity.

Rhenium isotopes have been under investigation for use in RIT. Both ^{186}Re and ^{188}Re have been linked to antibodies. ^{188}Re has the advantage of being easily generated with a ^{188}W generator. Its short half-life of 17 h renders it suitable for locoregional therapies or for therapies with small molecules that clear from the body rapidly. Because of its physical properties, including longer half-life, ^{186}Re has been more extensively studied. Rhenium nuclides have γ-emissions, which allow for easy detection of distribution and clearance by external scintigraphy. Early clinical trials have exploited the γ-emissions of ^{186}Re to analyze absorbed doses to tumors and normal organs and have demonstrated its safety for RIT of solid tumors (9).

Recent developments in radiochemistry have led to the increased interest in α-emitters for therapy. α-Particles are high-energy helium nuclei with high-linear-energy transfer. α-Particles have a range of only 50–80 μm, making them most useful for individual cells, such as micrometastases and circulating tumor cells. In fact, ^{213}Bi conjugated to a humanized anti-CD33 antibody is being evaluated in clinical trials for the treatment of advanced myelogenous leukemia. In addition to ^{212}Bi and ^{213}Bi, which are generated as eluates from ^{224}Ra and ^{225}Ac generators, cyclotron-produced ^{211}At is under investigation as therapy for several tumor types. Clinical trials have been reported using ^{211}At-labeled chimeric antitenascin antibody 81C6 for gliomas (10).

In vivo generators of α-particles are being investigated for their ability to deliver cytotoxic particles to micrometastases. These generators overcome the limitations posed

by the short half-lives of most α-particles. These "nanogenerators" have been studied in various tumor types in mice. One study conjugated a ^{225}Ac generator to an internalizing antibody that targeted Her-2/*neu* (trastuzumab) to study its efficacy in treating disease in a mouse model of advanced ovarian cancer (*11*). This generator produces 3 α-particle–emitting radioisotopes, including ^{213}Bi.

Auger emitters deposit high-linear-energy transfer over extremely short distances and are therefore most effective when the decay occurs in the nucleus and less so when the decay occurs in the cytoplasm (*12*). ^{125}I is the prototypical radionuclide, but its long half-life renders it less than optimal for therapy. Other similar radionuclides that have been studied, although not with antibodies, have included ^{111}In. In both cases, the amount of radioactivity necessary is economically prohibitive. A radionuclide that is gaining increasing attention in this category is ^{67}Ga. Improvements in chelation chemistry have resulted in stable radioimmunoconjugates with ^{67}Ga, and clinical trials are planned by us and several other groups.

RIT FOR SOLID TUMORS

Most clinical RIT studies on solid tumors have been performed on colon cancer. Secreted antigen systems such as carcinoembryonic antigen (CEA) and TAG-72 (sialyl Tn); cell surface antigens including 17-1A, Lewis Y (Le^y), and A33; and stromal antigens, notably fibroblast activation-protein-α, have been studied, both at this center and elsewhere. This section will highlight key phase 1 and 2 trials, particularly ones targeting colon cancer, as well as selected trials that are in early stages.

CEA

Starting with a radioiodinated polyclonal anti-CEA antibody (*13*), Goldenberg et al. pioneered the use of antibodies in the detection and therapy of cancer. This group has subsequently performed RIT studies with ^{131}I-labeled murine and humanized anti-CEA antibodies (*14–17*). A recent phase 2 trial of 21 patients treated with ^{131}I-labeled humanized anti-CEA mAb (hMN-14; Immunomedics, Inc.) studied response to RIT in both patients with small-volume metastasis refractory to treatment and patients who had undergone surgical resection of metachronous liver metastases with curative intent (*18*). Each patient received a single dose of 2,220 MBq/m^2 (60 mCi/m^2), which was previously demonstrated to be the maximum tolerated dose. Between both groups of patients, the study demonstrated an overall response rate of 58%, with a mean duration of response of 9 mo. Of the 9 patients receiving RIT in an adjuvant setting after surgery, 7 remained disease free at 36 mo after treatment. Although these results appear promising, they underscore the suitability of current methods of RIT for small-volume disease rather than bulky disease.

In another effort to develop nonimmunogenic antigen-binding constructs against CEA, Raubitschek's group at the City of Hope Medical Center have performed studies with a chimeric antibody. The initial dose-finding study established no mass dependence on tumor targeting and no evidence of immunogenicity (*19*). Subsequent RIT studies with ^{90}Y-labeled antibody (*20*) demonstrated that radionuclide toxicity was, again, nuclide dependent. No responses were observed in this nonmyeloablative trial, prompting a myeloablative trial (*21*), the final results of which are awaited. The observation that nonmyeloablative RIT with intact IgG is unlikely to result in major responses has spurred the development of various antigen-binding constructs (*22*), which are now being produced for clinical study.

Begent et al. have also performed studies using ^{131}I-labeled antigen-binding constructs (whole IgG, F(ab)$'_2$ fragments, cross-linked divalent and trivalent versions). Although there was selective targeting of iodinated sFv to tumor, the degree of uptake was perhaps not adequate to result in responses with ^{131}I (*23*). Begent et al. have continued their pioneering studies by developing a single-chain Fv fragment of their anti-CEA antibody. This sFv construct is grown in *E. coli* and exhibits impressive targeting abilities (*24*).

TAG-72

mAb CC49 is a murine IgG targeting the antigen (Tn/sialylTn) expressed on a tumor-associated mucin, TAG-72, that is expressed heterogeneously in most adenocarcinomas and thus is a potential target for RIT in several cancers. CC49 IgG exhibits high reactivity against tumor cells in most adenocarcinomas from colorectum, ovary, breast, stomach, and pancreas, with very little reactivity against normal tissues (*25*). After an initial study comparing CC49 with its lower-affinity counterpart B72.3 had shown CC49 to have better relative uptake in colorectal cancer (*26*), phase 1 studies with ^{131}I-CC49 in colorectal cancer demonstrated excellent tumor targeting and low levels of toxicity at a maximum tolerated dose (MTD) of 2,775 MBq/m^2 (75 mCi/m^2) (*27*). Further trials, however, have demonstrated tumor doses of 0.19–6.67 Gy (19–667 rad) but no objective tumor responses (*28*). Dose-limiting toxicity was hematopoietic and nuclide dependent. Moreover, in 12 of the 13 patients studied, human antimouse antibodies developed.

An attempt to reduce immunogenicity by administration of an immunosuppressive agent, deoxyspergualin, demonstrated less than satisfactory results (*29*), and so humanization of the antibody was embarked on. To ensure that clearance of the humanized antibody was as close as possible to that of the murine intact IgG, the CH$_2$ domain of the chimeric antibody was deleted. Initial clinical trials (*30*) have shown that clearance and targeting characteristics are indeed comparable and that the novel protein is nonimmunogenic. Retention of the CH$_3$ domain retains the immunobiologic functions of the antibody, and therefore a phase 1 trial to assess both the immunobiologic properties of CH$_2$-deleted huCC49 and the safety of ^{131}I-labeled antibody is under way.

The initial studies on colon cancer were followed by phase 1 and phase 2 studies on a variety of other solid tumors, notably breast and prostate cancers (30–33). Alvarez et al. performed a phase 1/2 trial of intraperitoneal ^{177}Lu-CC49 mAb in 27 patients with recurrent ovarian cancer (34). The MTD was 1,665 MBq/m^2 (45 mCi/m^2), and the dose-limiting toxicity was bone marrow suppression. In most patients with gross disease, the disease progressed after therapy. Prolonged disease-free survival was observed only in patients with microscopic disease. Since then, intraperitoneal RIT with ^{131}I-CC49 has also been performed on ovarian cancer (35) and combination therapy is being investigated (36).

In all these studies, there has been excellent tumor targeting but no significant clinical responses. As is the case with most antigen–antibody systems in solid tumors, the limitations in the clinical application of radiolabeled CC49 IgG are primarily due to normal tissue toxicity, immunogenicity, and relatively poor penetration into tumor. Genetically engineered sFvs are one way to potentially overcome some of these limitations. The sFvs had accelerated clearance from the vasculature, excellent penetration into the tumor from the vasculature, reduced immunogenicity, and higher tumor-to-background ratios than did corresponding IgG, F(ab′)$_2$, or Fab′ fragments in animal models (37–40). Early studies at this center by Larson et al. (41) of a ^{123}I-labeled sFv CC49 in human metastatic colorectal carcinoma demonstrated rapid distribution and clearance from the body via the kidneys and the feasibility of same-day imaging of the metastatic tumors in patients with colorectal carcinoma. However, because of their small size and monovalency, sFvs clear the body too rapidly to allow for sufficient tumor uptake and retention for therapeutic applications. Moreover, early sFvs were generated in bacterial systems and may not have been stable in vivo.

A33

A33 is a promising target for RIT in colon cancer. The A33 antigen, a transmembrane glycoprotein, is not secreted and is highly expressed by cells of colonic origin. In addition, mAb A33 is rapidly internalized into a cell after binding to the A33 antigen on the cell membrane. All these factors promote rapid tumor localization as well as high uptake by tumors. Phase 1/2 studies of ^{131}I-mAb A33 RIT were performed on patients with colon cancer (42). Again, dose-limiting toxicity was nuclide dependent, and the MTD was determined to be 2,775 MBq (75 mCi) of ^{131}I per square meter. Although the isotope showed variable uptake in normal bowel, gastrointestinal symptoms were mild or absent.

We also investigated ^{125}I-murine A33, as the radioiodinated antibody appears to not undergo catabolism after internalization (probably via the macropinosome). This phenomenon could provide a therapeutic advantage if antibodies internalize and therefore transport low-energy electron-emitting isotopes close to the tumor cell nucleus (43). A phase 1/2 study of ^{125}I-labeled mAb A33 demonstrated low rates of toxicity but only modest response rates (44). MTD was not reached with doses of up to 13 GBq/m^2 (350 mCi/m^2) of ^{125}I-mAb A33. Of the 21 patients, 1 demonstrated a mixed response based on imaging studies and 2 patients with stable disease demonstrated decreased serum CEA levels. Higher levels of response were seen in patients who pursued further chemotherapy (carmustine, vincristine, fluorouracil, and streptozocin) after RIT, suggesting a potential role of combined RIT and chemotherapy. Humanized A33 has been investigated by our group; it is less immunogenic and has a biodistribution profile comparable to that of the murine protein. Studies comparing the efficacy of combined radiosensitizing chemotherapy (capecitabine) and RIT with ^{131}I-huA33 are currently under way.

OTHER ANTIGENIC SYSTEMS STUDIED

Antibody 17-1A (murine IgG$_{2a}$), which reacts against the surface epithelial antigen KSA, has also been studied with different radionuclides. The internalizing properties of murine antibody 17-1A make it attractive as a carrier for ^{125}I. The antibody was chimerized, and a pilot clinical trial of increasing doses of ^{125}I-chimeric 17-1A in patients with metastatic colorectal cancer was conducted (45). This trial, performed at the University of Alabama in Birmingham, corroborated our results that high-dose outpatient RIT with a ^{125}I-labeled internalizing antibody could be achieved without significant patient toxicity or radiation hazard.

Our team has attempted to identify targets not only on cancer cell surfaces but also on other components of the tumor, including stroma and vasculature. We developed a novel targeting approach to colon cancer therapy by developing an antibody, F19, against fibroblast activation protein-α. Fibroblast activation protein-α is highly expressed by activated fibroblasts abundant in most solid tumors, including more than 95% of primary and metastatic colorectal carcinomas, but is not expressed by normal tissue (other than healing scars).

Further studies were performed to define the toxicity, imaging, and biodistribution characteristics of ^{131}I-labeled mAb F19. Because of selective localization in the tumor, with minimal uptake in the normal tissues, lesions as small as 1 cm could be visualized by scintigraphy (46). The easy accessibility of the fibroblast activation protein–positive tumor stromal fibroblasts to circulating mAb prompted the humanization of this antibody, now named sibrotuzumab. Phase 1 mass dose-finding studies have been completed with this novel protein (47), and RIT studies are being performed.

Ovarian carcinoma has a high mortality rate, because most ovarian carcinomas are detected at a late stage. In addition to CC49 antibodies, various other antibody–antigen systems have been studied in ovarian cancer. MX35 is expressed homogeneously in 90% of epithelial ovarian cancers and is also expressed in epithelial cells of the lung,

sweat glands, kidney, collecting ducts, thyroid, fallopian tube, cervix, and uterus (48). Several studies using [131]I- and ^{125}I-labeled mAb MX35 showed excellent localization to sites of ovarian tumors, including micrometastases in the peritoneal wall (49). MOv18 targets the membrane folate receptor that is highly expressed on ovarian carcinoma cells. [131]I chimeric MOv18 mAb was studied in 3 patients and showed moderate visualization of tumor sites, tumor-absorbed doses of 600–3,800 cGy, and stable disease for 2 to >6 mo (50). Moreover, administration of the antibody was not associated with development of human antichimeric antibodies. Intraperitoneal RIT using ^{90}Y-labeled murine anti-HMFG$_1$ (analogous to the *muc-1* antigen) as an adjuvant to chemotherapy compared with chemotherapy alone has shown increased overall survival in patients who received the RIT compared with those who received chemotherapy alone (51). Ovarian cancer is limited to the peritoneal cavity for most of its natural progression, and therefore intraperitoneal therapies have always held promise for a disease with limited treatment options.

Systemic RIT studies with anti-*muc1* antibodies have been performed largely on breast cancer. Unlike most of the studies above, which used radioiodine initially and then studied other radionuclides, the studies with a novel humanized anti-*muc1* antibody, BrE3, were performed with [90]Y (52). These studies showed that targeting using ^{111}In as a surrogate (53) was excellent and that phase 2 trials to establish efficacy were justified, especially since the humanized BrE3 antibody has immune effector function in vitro.

Limited studies with intrathecal RIT have also shown promise. Intrathecal RIT has potential, as radiation in the closed compartment is more likely to have efficacy when its retention can be enhanced by conjugation to a large molecule that will transit slowly into the systemic compartment. Most studies have been performed with β-minus–emitting nuclides (54–61), which may be less than ideal because the energy may be deposited outside the intrathecal space to varying extents depending on the nuclide. In this respect, α-emitters may be ideal, as they deposit high-linear-energy transfer radiation over submillimeter distances (62). No studies have progressed to phase 2 or higher; however, this is more a function of nuclide availability and patient characteristics than of lack of promise.

The Le^y antigen is a blood group–related antigen that is expressed in a high proportion of epithelial cancers, including breast, colon, ovarian, and lung cancer, and therefore is an attractive target for mAb-directed therapy. Early studies with anti-Le^y antibodies performed using chemoimmunoconjugates demonstrated significant gastrointestinal toxicity without significant efficacy (63). Pastan's group has studied another anti-Le^y antibody, labeled with either ^{111}In to study biodistribution or ^{90}Y for therapy (64). The phase 2 study demonstrated good tumor localization, and the MTD was found to be 740 MBq of [90]Y. Those investigators are now developing trials to study the efficacy of the radioimmunoconjugate in advanced gastrointestinal malignancy. They have also investigated an immunotoxin (65), and this holds promise for single-chain anti-Le^y proteins conjugated with radionuclides. The Le^y antigen is abundantly expressed in both gastric and ovarian cancers, which are limited to the peritoneal cavity for much of their natural course. We have therefore initiated a phase 1 intraperitoneal RIT study using ^{90}Y labeled to another humanized anti-Le^y antibody, hu3S193, initially generated at our center (66), in ovarian cancer patients with minimal residual disease (Fig. 1).

CARBONIC ANHYDRASE IX

Carbonic anhydrase IX is expressed in more than 80% of primary renal cell carcinomas and therefore is an excellent therapeutic target for a cancer refractory to chemotherapy and whose 5-y survival rate is less than 10%. Antibody G250, developed by Oosterwijk et al., recognizes carbonic anhydrase IX, with normal tissue cross-reactivity limited to biliary epithelium (67). An early study of mouse mAb G250 demonstrated excellent targeting to renal tumors, but treatment was limited by the development of a host immune response (68). This is particularly significant, as it precludes the use of multiple administrations, a method that is thought to be crucial for treatment of heterogeneous, bulky solid tumors. Recent phase 1 studies have used ^{131}I chimeric mAb G250 as a less immunogenic alternative to mG250. One such study examining distribution of antibody uptake in tumors used a diagnostic dose of [131]I cG250 before a single large radioimmunotherapeutic dose. To evaluate the effect of dose fractionation on safety and efficacy, we simultaneously started a single large-dose RIT study based on escalating amounts of ^{131}I and a study of fractionated cG250 using ≤1,110-MBq (30 mCi) ^{131}I-cG250 doses in a schema based on whole-body radiation-absorbed dose (69). The trial of fractionated cG250 demonstrated differential distribution of radioactivity after each treatment dose (Fig. 2). In this study, the MTD of ^{131}I was found to be 0.75 Gy and dose-limiting toxicity was hematologic. Preliminary calculations suggest that dose-limiting whole-body radiation-absorbed dose is not different in "rapidly" fractionated and single-large-dose treatment schemata (J.A. O'Donoghue and S.H. Bender, et al., unpublished data, July 2004).

Because the G250 antibody binds to epithelial cells of the large bile duct and gallbladder, and because biliary cancer is sensitive to radiation, RIT with cG250 may have clinical

FIGURE 1. Anterior (left) and posterior (right) images at various times after intraperitoneal administration of ^{111}In-labeled humanized anti-Le^y antibody hu3S193.
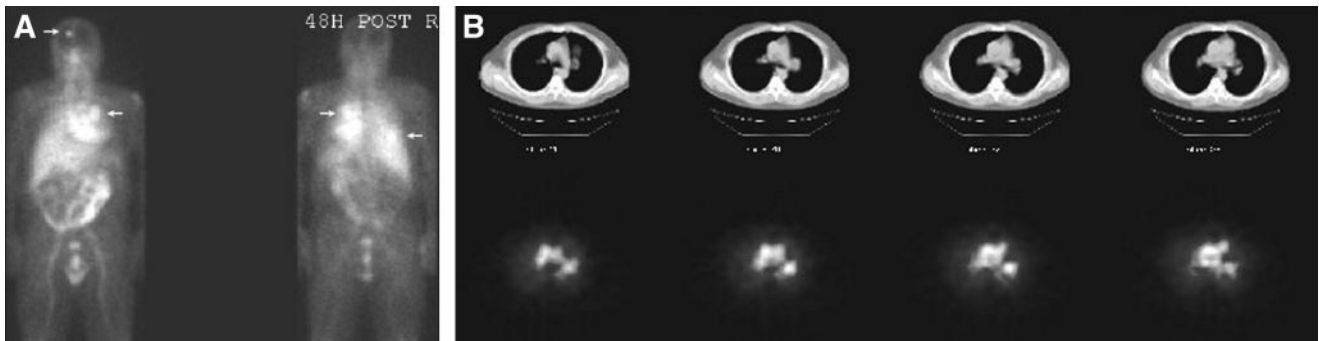
FIGURE 2. (A) Anterior (left) and posterior (right) whole-body images obtained 3 d after administration of ^{131}I-chimeric G250. Targeting to known lesions in scalp, hilum, and lung is excellent. (B) CT (top row) and SPECT (bottom row) coregistered images demonstrate uniform distribution throughout left hilar mass in same patient.

benefits in the treatment of biliary cancer patients. These cancers are particularly difficult to treat because of the late stage at diagnosis. The ^{131}I-labeled antibody WX-G250 RIT is designed to identify the tumor cells and deliver tumor-destroying doses of radiation directly to the tumor cells. Clinical phase 1/2 trials with WX-G250RIT in biliary cancer patients appear promising (*70*). Because the antigen is expressed on both normal biliary tissue and biliary cancers, the evaluation of differential expression is important to the selection of an appropriate RIT model, and we are therefore evaluating differential in vivo expression of tumor antigen expression using quantitative PET and ^{124}I-cG250.

PROSTATE-SPECIFIC-MEMBRANE ANTIGEN (PSMA)

Although prostate cancer has received much attention because of the success of diagnostic biomarkers, the success of RIT for the disease has lagged. Studies of ^{131}I-murine CC49 mAb failed to show an objective response (*71*). Subsequently, studies have attempted to increase antigen expression using interferon pretreatment (*72*). Fourteen patients received 7 doses of interferon followed by treatment with 2,775 MBq (75 mCi) of ^{131}I-CC49 per square meter. Although TAG-72 expression seemed to be upregulated, as evidenced by increased tumor localization, no significant response was achieved. Moreover, in 12 of the patients, human antimouse antibodies developed.

PSMA is not a secreted antigen and has been identified as an excellent target for RIT for prostate cancer. Studies of PSMA mAb 7E11/CY356 labeled with ^{90}Y demonstrated no therapeutic efficacy, probably because this antibody targets an internal epitope of PSMA, thus targeting only dead or dying tumor cells. In contrast, mAb J591 targets the external domain of PSMA (*73*). Phase 1 studies of ^{90}Y- and ^{177}Lu-labeled humanized J591 mAb are under way (*74*).

PRETARGETING STRATEGIES

To enhance the efficacy of RIT, multistep targeting strategies have been under development. These methods are designed to minimize the radiation to normal tissue that is due to a prolonged residence in the body. One approach uses the interaction of avidin and biotin. The targeting antibody is biotinylated before injection. After injection, avidin is injected in order to bind to the antibody in the tumor. Finally, radiolabeled biotin is injected and attaches to the avidin in the tumor. The use of a clearing agent may further reduce the amount of antibody in normal tissues (Fig. 3).

Early studies of pretargeted RIT designed to evaluate its safety and therapeutic ratio have demonstrated a mean tumor-to-marrow absorbed dose ratio of 63:1, which is an order of magnitude greater than the 6:1 ratio usually seen in conventional RIT (*75*). Further studies of pretargeted treatment with mAb NR-LU-10-streptavidin and ^{90}Y-biotin in refractory colorectal adenocarcinoma demonstrated a mean tumor absorbed dose of 0.5 ± 0.2 cGy/MBq, which was significantly higher than the dose estimates to the kidney (0.3 ± 0.1 cGy/MBq) and to the bone marrow (0.1 mGy/MBq). Despite these impressive ratios, only 8% of patients showed a major response to therapy. Moreover, although hematologic toxicity was not a limiting factor, nonhematologic toxicities were significant (*76*). It is anticipated that dose-limiting toxicity with most multistep techniques will not be hematologic but probably renal. Renal toxicity poses
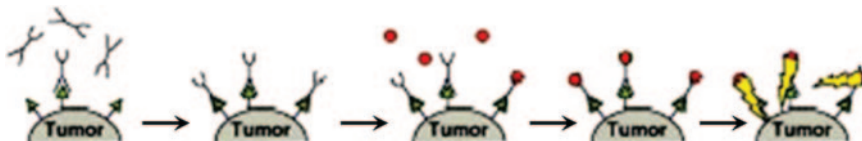

FIGURE 3. Tumor pretargeting with bispecific construct. Bispecific agent (e.g., bispecific antibody or antibody/streptavidin fusion) is administered and saturates antigenic sites on tumor. Subsequently, a small radionuclide carrier is administered and binds to the tumor-localizing bispecific agent.

additional issues, as nephropathy is usually not evident until several years after renal insult.

Promising results using the pretargeting approach have been seen in gliomas. Studies of biotinylated antitenascin mAb with ^{90}Y-1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid biotin as the radionuclide have demonstrated a response as the sole therapy for recurrent disease as well as in the adjuvant setting (*77*).

Bispecific constructs that react against a radiometal–chelate complex, on the other hand, are unlikely to be immunogenic and therefore hold promise as therapeutic agents. Studies are now being planned that would use these novel systems to deliver higher amounts of relative tumor radiation-absorbed dose than would be possible with single-step radiolabeled-antibody methods (*78,79*).

STRATEGIES FOR FUTURE STUDIES

The RIT studies described above demonstrate that the early lack of success in solid-tumor RIT has been an impetus for the development of a plethora of constructs linked to radionuclides with varying radiobiologic characteristics. Patient-based dosing, fractionated therapy, and multistep targeting are just some of the methods being investigated to optimize treatment. A new generation of intelligently designed clinical trials is beginning. These trials share some characteristics:

Advances in Radiochemistry

These have led to successful clinical trials of labeling Auger emitters and α-emitters to conjugates and pharmaceuticals resulting in stable compounds. Also, the development of positron emitters such as copper, yttrium, and iodine has the potential to allow PET-based dosimetry. These will permit calculation of tumor radiation-absorbed doses permitting accurate dose delivery. Although ^{131}I, ^{186}Re, and ^{188}Re are ideal radionuclides for external scintigraphy, surrogate γ-emitters must be used to evaluate the distribution and clearance of pure β-emitters. ^{111}In has been considered to be an appropriate surrogate for ^{90}Y. Their half-lives are almost identical, and both are readily incorporated into the same metal chelating agents. A recent study using PET to compare ^{86}Y and ^{111}In as surrogates for ^{90}Y showed that, although ^{111}In and ^{86}Y have similar biodistributions, ^{86}Y remained longer in organs such as bone (*80*). Given the slower clearance kinetics and bone-seeking properties of yttrium, ^{86}Y is a more suitable surrogate for ^{90}Y. This feature has been used to obtain extremely accurate dosimetry in bone-seeking radiopharmaceuticals (*81*) and in radiolabeled peptides (*82*).

Genetic Engineering

The development of nonimmunogenic antibody constructs, as well as pretargeting strategies such as an affinity-enhancement system, appears to enhance tumor-to-nontumor ratios and may increase radiation doses to tumor more selectively than do directly labeled antibodies. The pretar-geting studies described above demonstrate that although development of this strategy is still in the early stages, it holds great potential. Approaches to genetic modification of antigen-binding constructs to make them suitable for RIT have focused on decreasing size, usually entailing a change to univalency, as with single-chain constructs. Moreover, production in bacterial systems results in deglycosylated proteins that may thus have suboptimal affinity or avidity characteristics. Making bivalent diabodies has resulted in molecules that are significantly retained in the kidney (*83*), thus raising the specter of unacceptable nephrotoxicity. Several groups have attempted to retain bivalency and minimize renal accumulation by adding other moieties—notably, the CH_3 domain (*84*)—or adding cytotoxic agents such as tumor-necrosis factor dimers (*85*).

Understanding Radiobiology

Using radionuclides with physical characteristics tailored to the individual disease will improve our ability to treat tumors appropriately. Creating smart systemic targeted radiotherapy depends not only on carefully selecting appropriate antigen targets and antibody constructs but also on choosing radionuclides appropriate for the extent and type of disease. Choosing α-emitters for microscopic disease or energetic β-minus emitters for bulky disease, with use of emitter strengths that are proportional to disease bulk, will maximize efficacy while limiting side effects to normal tissue whenever possible. A prime concern with these multiple agents will be toxicity. It will therefore be important to combine nuclides with differing pharmacodynamic properties (e.g., combining bone-seeking β-minus–emitting ^{90}Y with ^{67}Ga, which has Auger emission and is cleared largely through the gastrointestinal tract) for therapy. The concept of sequential therapies with different nuclides based on tumor burden and other characteristics will also play a role in nuclide selection. There appears to be little doubt, however, that combination RIT will be as important to successful therapy as combination chemotherapy has been.

Combination Multimodality Therapy

Using chemotherapy and external-beam radiotherapy in conjunction with RIT earlier in the treatment of solid tumors has the potential to maximize the potential of RIT. Complementary modalities have great potential: A chemotherapeutic agent such as paclitaxel may not only have independent cytotoxicity but also act as a radiosensitizer and enhance the efficacy of RIT. We are investigating the potential of small-molecule inhibitors such as Iressa (Astra Zeneca) that can cause downstream metabolic effects changing tumor uptake of, as well as the susceptibility of tumor cells to, RIT. Agents that change the hypoxic fraction of tumors may also enhance RIT efficacy. Use of multiple modalities can cause changes in tumor permeability and vascularity that permit radioimmunoconjugates to access otherwise-unreachable tumor regions. The use of antiangiogenic agents is particularly of great interest: these agents (*86*) actually decrease tumor uptake of radioimmunoconju-

gate and, conversely, decrease egress of radioimmunoconjugate if the antiangiogenic agent is administered at an as yet undetermined time after RIT. These exciting studies are currently being designed in preclinical models and will soon be applied clinically.

Administration Schedule

Use of fractionated multidose RIT instead of single-larger-dose RIT may result in a slower rate of cell repopulation. Theoretic models have compared the effects of single-large-dose administration and rapid fractionation (*87*). Although a single large dose may produce a high rate of cell killing, fractionated therapy offers the advantages of lower toxicity and prolonged tumor response. In addition, similar to the rationale behind multimodality therapy, preceding doses may cause architectural changes in the tumor that may allow subsequent doses to target previously inaccessible regions. However, our studies with fractionated and single-large-dose RIT have shown no advantage to safety or improvement in total tumor radiation-absorbed dose. We are currently analyzing the data to determine whether the intratumoral distribution of radioactivity has changed (*69*). Nevertheless, fractionation may have promise when combined in a multimodality therapeutic strategy.

These strategies will pave the way toward tailored RIT for solid tumors that is both safe and effective. After the success of RIT for hematologic cancers, there is little doubt that RIT will find its niche in cancer therapy, both in the adjuvant situation and in bulky disease. Because cancer therapy must target both isolated cancer cells and bulky tumors, combination RIT, given either sequentially or simultaneously, will soon be a crucial player in the era of molecular therapeutics.

CONCLUSION

At a time when molecular medicine is becoming a reality, RIT has already proven its role in the treatment of non-Hodgkin's lymphoma and is poised to find a role in the treatment of solid tumors. Humanized and other nonimmunogenic antigen-binding constructs by themselves are finding their place in cancer therapy, as are small molecules that modulate a variety of processes from cell surface receptor expression to intracellular enzyme activity. The toxicity inherent in radioactivity will dictate that the role of RIT eventually be weighed against the benefits and side effects of a multitude of targeted molecular therapies. Nevertheless, the result may well be treatments that exploit multiple cellular processes sequentially, each rendering the tumor more amenable to treatment by the next, with RIT finding a place in the vast new armamentarium that awaits the future of targeted therapy.

REFERENCES

1. Witzig TE, White CA, Gordon LI, et al. Safety of yttrium-90 ibritumomab tiuxetan radioimmunotherapy for relapsed low-grade, follicular, or transformed non-Hodgkin's lymphoma. *J Clin Oncol.* 2003;21:1263–1270.

2. Horning SJ. Future directions in radioimmunotherapy for B-cell lymphoma. *Semin Oncol.* 2003;30(suppl):29–34.

3. Press OW. Radioimmunotherapy for non-Hodgkin's lymphomas: a historical perspective. *Semin Oncol.* 2003;30(suppl):10–21.

4. Press OW, Unger JM, Braziel RM, et al. A phase 2 trial of CHOP chemotherapy followed by tositumomab/iodine I 131 tositumomab for previously untreated follicular non-Hodgkin lymphoma: Southwest Oncology Group Protocol S9911. *Blood.* 2003;102:1606–1612.

5. Kaminski MS, Zelenetz AD, Press OW, et al. Pivotal study of iodine I 131 tositumomab for chemotherapy-refractory low-grade or transformed low-grade B-cell non-Hodgkin's lymphomas. *J Clin Oncol.* 2001;19:3918–3928.

6. O'Donoghue JA. The implications of non-uniform tumor doses for radioimmunotherapy. *J Nucl Med.* 1999;40:1337–1341.

7. Schlom J, Molinolo A, Simpson JF, et al. Advantage of dose fractionation in monoclonal antibody-targeted radioimmunotherapy. *J Natl Cancer Inst* 1990;82:763–771.

8. Zanzonico PB, Siegel JA, St Germain J. A generalized algorithm for determining the time of release and the duration of post-release radiation precautions following radionuclide therapy. *Health Phys.* 2000;78:648–659.

9. Postema EJ, Borjesson PK, Buijs WC, et al. Dosimetric analysis of radioimmunotherapy with ^{186}Re-labeled bivatuzumab in patients with head and neck cancer. *J Nucl Med.* 2003;44:1690–1699.

10. Zalutsky M, Reardon D, Akabani G, et al. Astatine-211 labeled human/mouse chimeric anti-tenascin monoclonal antibody via surgically created resection cavities for patients with recurrent gliomas: phase I study [abstract]. *Neuro Oncol.* 2002;4(suppl):S103.

11. Borchardt PE, Yuan RR, Miederer M, McDevitt MR, Scheinberg DA. Targeted actinium-225 in vivo generators for therapy of ovarian cancer. *Cancer Res.* 2003;63:5084–5090.

12. Adelstein SJ, Kassis AI, Bodei L, Mariani G. Radiotoxicity of iodine-125 and other auger-electron-emitting radionuclides: background to therapy. *Cancer Biother Radiopharm.* 2003;18:301–316.

13. Goldenberg DM, DeLand F, Kim E, et al. Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. *N Engl J Med.* 1978;298:1384–1386.

14. Juweid M, Sharkey RM, Swayne LC, Griffiths GL, Dunn R, Goldenberg DM. Pharmacokinetics, dosimetry and toxicity of rhenium-188-labeled anti-carcinoembryonic antigen monoclonal antibody, MN-14, in gastrointestinal cancer. *J Nucl Med.* 1998;39:34–42.

15. Behr TM, Sharkey RM, Juweid ME, et al. Phase I/II clinical radioimmunotherapy with an iodine-131-labeled anti-carcinoembryonic antigen murine monoclonal antibody IgG. *J Nucl Med.* 1997;38:858–870.

16. Juweid ME, Sharkey RM, Behr T, et al. Radioimmunotherapy of patients with small-volume tumors using iodine-131-labeled anti-CEA monoclonal antibody NP-4 F(ab′)2. *J Nucl Med.* 1996;37:1504–1510.

17. Juweid M, Sharkey RM, Behr TM, et al. Clinical evaluation of tumor targeting with the anticarcinoembryonic antigen murine monoclonal antibody fragment, MN-14 F(ab)2. *Cancer.* 1996;78:157–168.

18. Behr TM, Liersch T, Greiner-Bechert L, et al. Radioimmunotherapy of small-volume disease of metastatic colorectal cancer. *Cancer.* 2002;94(suppl):1373–1381.

19. Wong JY, Chu DZ, Yamauchi D, et al. Dose escalation trial of indium-111-labeled anti-carcinoembryonic antigen chimeric monoclonal antibody (chimeric T84.66) in presurgical colorectal cancer patients. *J Nucl Med.* 1998;39:2097–2104.

20. Wong JYC, Chu DZ, Yamauchi DM, et al. A phase I radioimmunotherapy trial evaluating 90yttrium-labeled anti-carcinoembryonic antigen (CEA) chimeric T84.66 in patients with metastatic CEA-producing malignancies. *Clin Cancer Res.* 2000;6:3855–3863.

21. Wong JY, Somlo G, Odom-Maryon T, et al. Initial clinical experience evaluating yttrium-90-chimeric T84.66 anticarcinoembryonic antigen antibody and autologous hematopoietic stem cell support in patients with carcinoembryonic antigen-producing metastatic breast cancer. *Clin Cancer Res.* 1999;5(suppl):3224s–3231s.

22. Wu AM, Yazaki PJ, Tsai S, et al. High-resolution microPET imaging of carcinoembryonic antigen-positive xenografts by using a copper-64-labeled engineered antibody fragment. *Proc Natl Acad Sci USA.* 2000;97:8495–8500.

23. Begent RH, Ledermann JA, Green AJ, et al. Antibody distribution and dosimetry in patients receiving radiolabelled antibody therapy for colorectal cancer. *Br J Cancer.* 1989;60:406–412.

24. Begent RH, Verhaar MJ, Chester KA, et al. Clinical evidence of efficient tumor targeting based on single-chain Fv antibody selected from a combinatorial library. *Nat Med.* 1996;2:979–984.

25. Molinolo A, Simpson JF, Thor A, Schlom J. Enhanced tumor binding using

immunohistochemical analyses by second generation anti-tumor-associated gly-coprotein 72 monoclonal antibodies versus monoclonal antibody B72.3 in human tissue. *Cancer Res.* 1990;50:1291–1298.

26. Divgi CR, Scott AM, McDermott K, et al. Clinical comparison of radiolocalization of two monoclonal antibodies (mAbs) against the TAG-72 antigen. *Nucl Med Biol.* 1994;21:9–15.

27. Divgi CR, Scott AM, Dantis L, et al. Phase I radioimmunotherapy trial with iodine-131-CC49 in metastatic colon carcinoma. *J Nucl Med.* 1995;36:586–592.

28. Murray JL, Macey DJ, Kasi LP, et al. Phase II radioimmunotherapy trial with ^{131}I-CC49 in colorectal cancer. *Cancer.* 1994;73(suppl):1057–1066.

29. Divgi CR, Scott AM, Gulec S, et al. Pilot radioimmunotherapy trial with ^{131}I-labeled murine monoclonal antibody CC49 and deoxyspergualin in metastatic colon carcinoma. *Clin Cancer Res.* 1995;1:1503–1510.

30. Macey DJ, Grant EJ, Kasi L, et al. Effect of recombinant alpha-interferon on pharmacokinetics, biodistribution, toxicity, and efficacy of ^{131}I-labeled monoclonal antibody CC49 in breast cancer: a phase II trial. *Clin Cancer Res.* 1997;3:1547–1555.

31. Meredith RF, Khazaeli MB, Macey DJ, et al. Phase II study of interferon-enhanced ^{131}I-labeled high affinity CC49 monoclonal antibody therapy in patients with metastatic prostate cancer. *Clin Cancer Res* 1999;5(suppl):3254s–3258s.

32. Meredith RF, Khazaeli MB, Plott WE, et al. Phase II study of dual ^{131}I-labeled monoclonal antibody therapy with interferon in patients with metastatic colorectal cancer. *Clin Cancer Res.* 1996;2:1811–1818.

33. Mulligan T, Carrasquillo JA, Chung Y, et al. Phase I study of intravenous Lu-labeled CC49 murine monoclonal antibody in patients with advanced adenocarcinoma. *Clin Cancer Res.* 1995;1:1447–1454.

34. Alvarez RD, Partridge EE, Khazaeli MB, et al. Intraperitoneal radioimmunotherapy of ovarian cancer with ^{177}Lu-CC49: a phase I/II study. *Gynecol Oncol.* 1997;65:94–101.

35. Meredith RF, Alvarez RD, Partridge EE, et al. Intraperitoneal radioimmunochemotherapy of ovarian cancer: a phase I study. *Cancer Biother Radiopharm.* 2001;16:305–315.

36. Alvarez RD, Huh WK, Khazaeli MB, et al. A phase I study of combined modality (90)yttrium-CC49 intraperitoneal radioimmunotherapy for ovarian cancer. *Clin Cancer Res.* 2002;8:2806–2811.

37. Colcher D, Bird R, Roselli M, et al. In vivo tumor targeting of a recombinant single-chain antigen-binding protein. *J Natl Cancer Inst.* 1990;82:1191–1197.

38. Milenic DE, Yokota T, Filpula DR, et al. Construction, binding properties, metabolism, and tumor targeting of a single-chain Fv derived from the pancarcinoma monoclonal antibody CC49. *Cancer Res.* 1991;51:6363–6371.

39. Adams GP, McCartney JE, Tai MS, et al. Highly specific in vivo tumor targeting by monovalent and divalent forms of 741F8 anti-c-erbB-2 single-chain Fv. *Cancer Res.* 1993;53:4026–4034.

40. Yokota T, Milenic DE, Whitlow M, Wood JF, Hubert SL, Schlom J. Micro autoradiographic analysis of the normal organ distribution of radioiodinated single-chain Fv and other immunoglobulin forms. *Cancer Res.* 1993;53:3776–3783.

41. Larson SM, El-Shirbiny AM, Divgi CR, et al. Single chain antigen binding protein (scFv CC49): first human studies in colorectal carcinoma metastatic to liver. *Cancer.* 1997;80:2458–2468.

42. Welt S, Divgi CR, Kemeny N, et al. Phase I/II study of iodine 131-labeled monoclonal antibody A33 in patients with advanced colon cancer. *J Clin Oncol.* 1994;12:1561–1571.

43. Daghighian F, Barendswaard E, Welt S, et al. Enhancement of radiation dose to the nucleus by vesicular internalization of iodine-125-labeled A33 monoclonal antibody. *J Nucl Med.* 1996;37:1052–1057.

44. Welt S, Scott AM, Divgi CR, et al. Phase I/II study of iodine 125-labeled monoclonal antibody A33 in patients with advanced colon cancer. *J Clin Oncol.* 1996;14:1787–1797.

45. Meredith RF, Khazaeli MB, Plott WE, et al. Initial clinical evaluation of iodine-125-labeled chimeric 17-1A for metastatic colon cancer. *J Nucl Med.* 1995;36:2229–2233.

46. Welt S, Divgi CR, Scott AM, et al. Antibody targeting in metastatic colon cancer: a phase I study of monoclonal antibody F19 against a cell-surface protein of reactive tumor stromal fibroblasts. *J Clin Oncol.* 1994;12:1193–1203.

47. Scott AM, Wiseman G, Welt S, et al. A phase I dose-escalation study of sibrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. *Clin Cancer Res.* 2003;9:1639–1647.

48. Rubin SC, Kostakoglu L, Divgi C, et al. Biodistribution and intraoperative evaluation of radiolabeled monoclonal antibody MX35 in patients with epithelial ovarian cancer. *Gynecol Oncol.* 1993;51:61–66.

49. Finstad CL, Lloyd KO, Federici MG, et al. Distribution of radiolabeled monoclonal antibody MX35 F(ab′)2 in tissue samples by storage phosphor screen

50. image analysis: evaluation of antibody localization to micrometastatic disease in epithelial ovarian cancer. *Clin Cancer Res.* 1997;8:1433–1442.

50. van Zanten-Przybysz I, Molthoff CF, Roos JC, et al. Radioimmunotherapy with intravenously administered ^{131}I-labeled chimeric monoclonal antibody MOv18 in patients with ovarian cancer. *J Nucl Med.* 2000;41:1168–1176.

51. Nicholson S, Gooden CS, Hird V, et al. Radioimmunotherapy after chemotherapy compared to chemotherapy alone in the treatment of advanced ovarian cancer: a matched analysis. *Oncol Rep.* 1998;5:223–226.

52. DeNardo SJ, Kramer EL, O'Donnell RT, et al. Radioimmunotherapy for breast cancer using indium-111/yttrium-90 BrE-3: results of a phase I clinical trial. *J Nucl Med.* 1997;38:1180–1185.

53. Kramer EL, Liebes L, Wasserheit C, et al. Initial clinical evaluation of radiolabeled MX-DTPA humanized BrE-3 antibody in patients with advanced breast cancer. *Clin Cancer Res.* 1998;4:1679–1688.

54. Richardson RB, Kemshead JT, Davies AG, et al. Dosimetry of intrathecal iodine131 monoclonal antibody in cases of neoplastic meningitis. *Eur J Nucl Med.* 1990;17:42–48.

55. Moseley RP, Davies AG, Richardson RB, et al. Intrathecal administration of ^{131}I radiolabelled monoclonal antibody as a treatment for neoplastic meningitis. *Br J Cancer.* 1990;62:637–642.

56. Hopkins K, Kemshead JT. Progress review: intrathecal and intratumoral injection of radiolabeled monoclonal antibodies (MoAbs) for the treatment of central nervous system (CNS) malignancies. *J Drug Target.* 1993;1:175–183.

57. Bigner DD, Brown M, Coleman RE, et al. Phase I studies of treatment of malignant gliomas and neoplastic meningitis with ^{131}I-radiolabeled monoclonal antibodies anti-tenascin 81C6 and anti-chondroitin proteoglycan sulfate Me1-14 F (ab′)2: a preliminary report. *J Neurooncol.* 1995;24:109–122.

58. Papanastassiou V, Pizer BL, Chandler CL, Zananiri TF, Kemshead JT, Hopkins KI. Pharmacokinetics and dose estimates following intrathecal administration of ^{131}I-monoclonal antibodies for the treatment of central nervous system malignancies. *Int J Radiat Oncol Biol Phys.* 1995;31:541–552.

59. Kemshead JT, Hopkins KI, Chandler CL. Treatment of diffuse leptomeningeal malignancy by intrathecal injection of ^{131}I radioimmunoconjugates: recent results. *Cancer Res.* 1996;141:145–158.

60. Brown MT, Coleman RE, Friedman AH, et al. Intrathecal ^{131}I-labeled antitenascin monoclonal antibody 81C6 treatment of patients with leptomeningeal neoplasms or primary brain tumor resection cavities with subarachnoid communication: phase I trial results. *Clin Cancer Res.* 1996;2:963–972.

61. Kramer K, Cheung NK, Humm JL, et al. Targeted radioimmunotherapy for leptomeningeal cancer using (131)I-3F8. *Med Pediatr Oncol.* 2000;35:716–718.

62. Zalutsky MR, Stabin MG, Larsen RH, Bigner DD. Tissue distribution and radiation dosimetry of astatine-211-labeled chimeric 81C6, an alpha-particle-emitting immunoconjugate. *Nucl Med Biol.* 1997;24:255–261.

63. Saleh MN, Sugarman S, Murray J, et al. Phase I trial of the anti-Lewis Y drug immunoconjugate BR96-doxorubicin in patients with Lewis Y-expressing epithelial tumors. *J Clin Oncol.* 2000;18:2282–2292.

64. Pai-Scherf LH, Carrasquillo JA, Paik C, et al. Imaging and phase I study of [111]In- and ^{90}Y-labeled anti-LewisY B3. *Clin Cancer Res.* 2000;6:1720–1730.

65. Pai LH, Wittes R, Setser A, Willingham MC, Pastan I. Treatment of advanced solid tumors with immunotoxin LMB-1: an antibody linked to Pseudomonas exotoxin. *Nat Med.* 1996;2:350–353.

66. Kitamura K, Stockert E, Garin-Chesa P, et al. Specificity analysis of blood group Lewis-y (Le(y)) antibodies generated against synthetic and natural Le(y) determinants. *Proc Natl Acad Sci USA.* 1994;91:12957–12961.

67. Oosterwijk E, Bander NH, Divgi CR, et al. Antibody localization in human renal cell carcinoma: a phase I study of monoclonal antibody G250. *J Clin Oncol.* 1993;11:738–750.

68. Divgi CR, Bander NH, Scott AM, et al. Phase I/II radioimmunotherapy trial with iodine-131-labeled monoclonal antibody G250 in metastatic renal cell carcinoma. *Clin Cancer Res.* 1998;4:2729–2739.

69. Divgi CR, O'Donoghue JA, Welt S, et al. Phase I clinical trial with fractionated radioimmunotherapy using ^{131}I-labeled chimeric G250 in metastatic renal cancer. *J Nucl Med.* 2004;45:1412–1421.

70. Summary of the G250 program. Wilex AG Web site. Available at: http://www.wilex.de/mains/rad/g250.htm. Accessed August 4, 2004.

71. Meredith RF, Bueschen AJ, Khazaeli MB, et al. Treatment of metastatic prostate carcinoma with radiolabeled antibody CC49. *J Nucl Med.* 1994;35:1017–1022.

72. Slovin SF, Scher HI, Divgi CR, et al. Interferon-gamma and monoclonal antibody ^{131}I-labeled CC49: outcomes in patients with androgen-independent prostate cancer. *Clin Cancer Res.* 1998;4:643–651.

73. Bander NH, Nanus DM, Milowsky MI, Kostakoglu L, Vallabahajosula S, Goldsmith SJ. Targeted systemic therapy of prostate cancer with a monoclonal antibody to prostate-specific membrane antigen. *Semin Oncol.* 2003;30:667–676.

74. Nanus DM, Milowsky MI, Kostakoglu L, et al. Clinical use of monoclonal

antibody HuJ591 therapy: targeting prostate specific membrane antigen. *J Urol.* 2003;170:S84–S88.

75. Breitz HB, Weiden PL, Beaumier PL, et al. Clinical optimization of pretargeted radioimmunotherapy with antibody-streptavidin conjugate and ^{90}Y-DOTA-biotin. *J Nucl Med.* 2000;41:131–140.

76. Breitz H, Knox S, Widen P, et al. Pretargeted radioimmunotherapy with antibody-streptavidin and Y-90 DOTA-biotin (Avicidin): result of a dose escalation study [abstract]. *J Nucl Med.* 1998;39(suppl):71P

77. Paganelli G, Bartolomei M, Ferrari M, et al. Pre-targeted locoregional radioimmunotherapy with ^{90}Y-biotin in glioma patients: phase I study and preliminary therapeutic results. *Cancer Biother Radiopharm.* 2001;16:227–235.

78. Sharkey RM, McBride WJ, Karacay H, et al. A universal pretargeting system for cancer detection and therapy using bispecific antibody. *Cancer Res.* 2003;63: 354–363.

79. Bardies M, Bardet S, Faivre-Chauvet A, et al. Bispecific antibody and iodine-131-labeled bivalent hapten dosimetry in patients with medullary thyroid or small-cell lung cancer. *J Nucl Med.* 1996;37:1853–1859.

80. Lovqvist A, Humm JL, Sheikh A, et al. PET imaging of (86)Y-labeled anti-Lewis Y monoclonal antibodies in a nude mouse model: comparison between (86)Y and (111)In radiolabels. *J Nucl Med.* 2001;42:1281–1287.

81. Rosch F, Herzog H, Plag C, et al. Radiation doses of yttrium-90 citrate and yttrium-90 EDTMP as determined via analogous yttrium-86 complexes and positron emission tomography. *Eur J Nucl Med.* 1996;23:958–966.

82. Jamar F, Barone R, Mathieu I, et al. ^{86}Y-DOTA(0)-D-Phe1-Tyr3-octreotide (SMT487): a phase 1 clinical study—pharmacokinetics, biodistribution and renal protective effect of different regimens of amino acid co-infusion. *Eur J Nucl Med Mol Imaging.* 2003;30:510–518.

83. Yoo TM, Chang HK, Choi CW, et al. Technetium-99m labeling and biodistribution of anti-TAC disulfide-stabilized Fv fragment[b]. *J Nucl Med.* 1997;38: 294–300.

84. Wu AM. Engineering multivalent antibody fragments for in vivo targeting. *Methods Mol Biol.* 2004;248:209–225.

85. Bauer S, Adrian N, Williamson B, et al. Targeted bioactivity of membrane-anchored TNF by an antibody-derived TNF fusion protein. *J Immunol.* 2004; 172:3930–3939.

86. Thorpe PE. Vascular targeting agents as cancer therapeutics. *Clin Cancer Res.* 2004;10:415–427.

87. DeNardo GL, Schlom J, Buchsbaum DJ, et al. Rationales, evidence, and design considerations for fractionated radioimmunotherapy. *Cancer.* 2002;94(4 suppl): 1332–1348.